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Article

Antiproliferative Activity of New Pyrazole-4-sulfonamide Derivatives: Synthesis and Biological Evaluation

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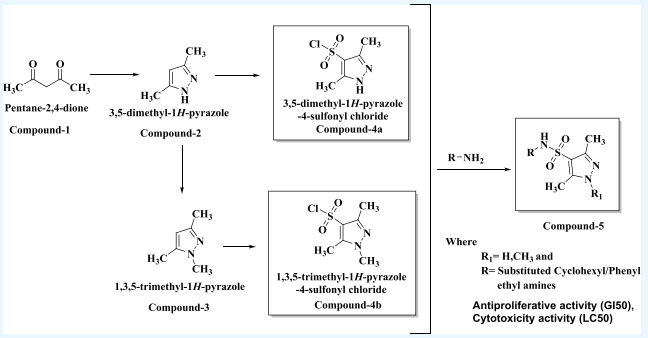
ABSTRACT: Pyrazole and sulfonamide constitute an important class of drugs, with several types of pharmacological agents. Facile synthesis of two new series of 3,5-dimethyl-1*H*-pyrazole-4-sulfonamide and 1,3,5-trimethyl-1*H*-pyrazole-4-sulfonamide derivatives was designed and synthesized. These pyrazole-4-sulfonamide derivatives are characterized by Fourier transform infrared (FT-IR), ^{1}H NMR, ^{13}C NMR, and elemental analysis, and their biological evolution data are presented. This paved way for the development of new pyrazole-4-sulfonamide derivatives. These compounds are tested for their in vitro antiproliferative activity against U937 cells by the CellTiter-Glo Luminescent cell viability assay using Mitomycin C. Cytotoxicity detection is based on the measurement of LDH activity, while these compounds did not exhibit cytotoxic activity on these cells. Half maximal inhibitory concentration (IC_{50}) was calculated by Graph Pad Prism software for each dose. Their structure−activity relationships were obtained and discussed.



INTRODUCTION

Heterocyclic compounds are a unique class of compounds that make up more than half of all known organic compounds, and they have a wide range of physical, chemical, and biological properties. Heterocyclic compounds are omnipresent. They are present in pharmaceuticals, agrochemicals, dyes, and many others. In addition to naturally occurring compounds, many synthetic heterocyclic compounds with several physiological and pharmacological properties are also known.[1−5]

The term pyrazole was given by Ludwig Knorr in 1883. Pyrazole refers to a class of simple aromatic ring compounds of the heterocyclic series characterized by a five-membered ring structure composed of three carbon atoms and two nitrogen atoms in the adjacent positions.

Among the two nitrogen atoms, one is basic and the other is neutral in nature. These are aromatic molecules due to their planar conjugated ring structures with six delocalized π-electrons. The partially reduced forms of pyrazole are named pyrazolines while the completely reduced form is pyrazolidine. Various access routes to the pyrazole nucleus have undergone numerous modifications since the first synthesis described by Knorr.

The presence of the pyrazole and sulfonamide nucleus in different structures leads to diversified applications in different areas such as technology, medicine, and agriculture. The pyrazole sulfonamide moiety is a prominent structural motif and serves as a pharmacophore in numerous pharmaceutically active compounds.[6−9] Sulfonamide derivatives are disclosed as anti-inflammatories in WO 2004/019935 and WO 2004/050631. Pharmaceutically active sulfonamides are also disclosed in *Arch. Pharm.* **1980**, *313*, 166−173, *J. Med. Chem.* 2003, *46*, 64−73, *J. Med. Chem.* 1997, *40*, 996−1004, EP 0031954, EP 1190710 (WO 200124786), US 5861401, US 4948809, US 6323199, US 3992441, and WO 99/33786.

In particular, they are described as inhibitors of protein glycation, antibacterial, antifungal, anticancer, antidepressant, anti-inflammatory, anti-tuberculosis, anti-obesity, antibacterial, antimicrobial, antifungal, antioxidant, as well as antiviral agents (Figure 1).

Pyrazole sulfonamides diversely substituted by aromatic and heteroaromatic groups possess numerous biological activities, which makes them particularly interesting.

In synthetic organic chemistry, the evolution of novel approaches for the preparation of pyrazole with sulfonamide derivatives along with their bioactivity's examination is known to be an important and it is a continuing challenge. Pyrazole-4-sulfonamide moieties containing active compounds are very

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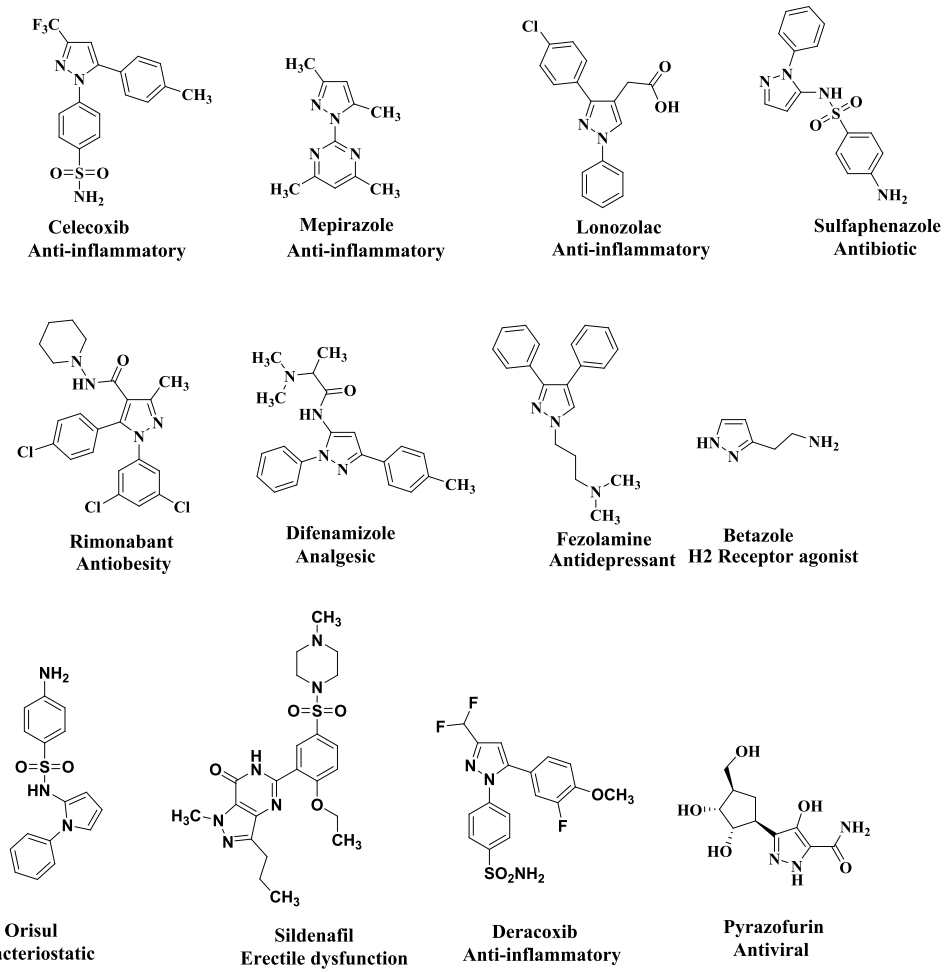

Figure 1. Pharmaceutical drugs in the market with pyrazole heterocyclic and sulfonamide moieties.

limited and their biological activity is still under investigation. Herein, we report the two new series of pyrazole-4-sulfonamide derivative synthesis, characterization, and their biological evolution.

EXPERIMENTAL CONDITIONS

Synthesis of 3,5-Dimethyl-1*H*-pyrazole. 3,5-Dimethyl-1*H*-pyrazole was synthesized by coupling pentane-2,4-dione with 85% hydrazine hydrate in methanol at 25−35 °C. It is an exothermic reaction that gives 3,5-dimethyl-1*H*-pyrazole quantitatively.

Synthesis of 1,3,5-Trimethyl-1*H*-pyrazole. To a clear solution of 3,5-dimethyl-1*H*-pyrazole (30 g, 312 mmol) in 210 mL of THF at 0 °C, potassium *tert*-butoxide (63 g, 561.7 mmol) was added in small portions under nitrogen atmosphere. After the completion of addition, the reaction mass was stirred at 25−30 °C about 40 min. Methyl iodide (57.6 g, 405.7 mmol) in 90 mL of THF was added to the reaction mass at 25−30 °C over a period of 30 min under a nitrogen atmosphere. After the completion of addition, the reaction mass was stirred at 25−30 °C for 16 h. The course of reaction was followed by thin-layer chromatography TLC. After the completion of the reaction, cold water was added followed by ethyl acetate. The contents were stirred for 10 min, and the upper organic layer was separated. The aqueous layer was again extracted with ethyl acetate. The combined organic layers were washed with water and dried over sodium sulfate and evaporated under vacuum to obtain 1,3,5-trimethyl-1*H*-pyrazole, yield: 27 g, 78%.

Sulfonylation. Sulfonylation on 3,5-dimethyl-1*H*-pyrazole and 1,3,5-trimethyl-1*H*-pyrazole was done by taking the respective pyrazole compound (25 g, 260 mmol) in 75 mL of chloroform. This mixture was added to a stirred solution of chlorosulfonic acid (166.7 g, 1430 mmol) in 175 mL of chloroform under a nitrogen atmosphere at 0 °C very slowly. The reaction mass temperature was raised to 60 °C and stirring was continued for 10 h. To this reaction mass was added thionyl chloride (40.8 g, 343.2 mmol) at 60 °C over 20 min. This reaction was further stirred for 2 h at 60 °C. The course of reaction was followed by TLC. After the completion of the reaction, the reaction mass was cooled to 0−10 °C and the reaction mass was added to a mixture of dichloromethane and ice-cold water. The lower organic layer was separated, dried over sodium sulfate, and evaporated under vacuum to obtain 3,5-dimethyl-1*H*-pyrazolesulfonyl chloride and 1,3,5-trimethyl-1*H*-pyrazolesulfonyl chloride (Scheme 1).[10−14]

Synthesis of Pyrazole Sulfonamide. 2-Phenylethylamine (65.5 mg, 2.7 mmol) was taken in 5 vol of dichloromethane and added diisopropylethylamine (99.6 mg, 3.85 mmol) at 25−30 °C. Pyrazole-4-sulfonyl chloride (100 mg, 2.57 mmol) in 5 vol of dichloromethane was added at 25−30 °C to the above reaction mixture. This reaction mass was stirred for 16 h at 25−30 °C. The course of reaction was monitored by TLC. After completion of reaction, 10 vol of cold water was added to

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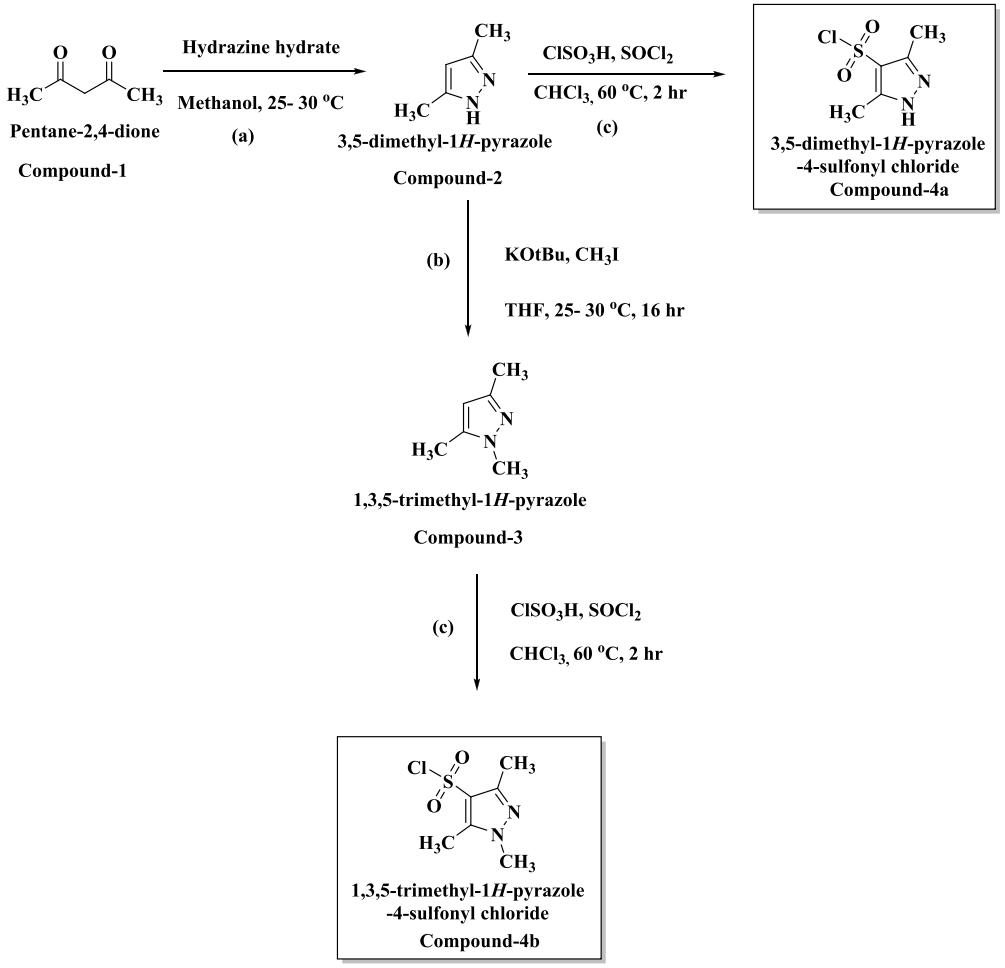
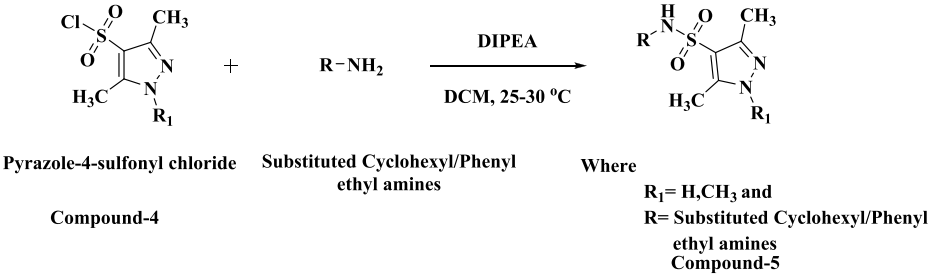
Scheme 1. Synthesis of 3,5-Dimethyl-1*H*-pyrazole-4-sulfonyl Chloride and 1,3,5-Trimethy-1*H*-pyrazole-4-sulfonyl Chloride[a]



[a]Reagents and conditions: (a) 85% hydrazine hydrate, methanol, 25−30 °C, yield 95%; (b) potassium *tert*-butoxide, CH_3I, THF, yield 78%; (c) chlorosulfonic acid, $SOCl_2$, $CHCl_3$, 60 °C, yield 90%.

Scheme 2. Synthesis of 3,5-Dimethyl-*N*-phenethyl-1*H*-pyrazole-4-sulfonamide and 1,3,5-Trimethyl-*N*-phenethyl-1*H*-pyrazole-4-sulfonamide



the reaction mass and stirred for 10 min. The lower organic layer was separated, and it was dried over sodium sulfate and evaporated under vacuum to obtain the crude compound. This crude compound was purified by column chromatography to give pure pyrazole-4-sulfonamide[15] compound.

3,5-dimethyl-1*H*-Pyrazole-4-sulfonamide and 1,3,5-trimethyl-1*H*-pyrazole-4-sulfonamide derivatives were prepared by reacting the appropriate Pyrazole sulfonyl chloride with different 2-phenylethylamine derivatives (Scheme 2).

Among the different reagents, potassium *tert*-butoxide in THF was obtained in good yield (Table 1: entry 13, 78%). In NaH and DMF, the reaction was completed in 12 h, but in TLC, a series of spots appeared when compared to THF (Table 1: entry 12, 55%). Potassium *tert*-butoxide is a better base compared to NaH, NaOH, Na_2CO_3, and K_2CO_3.

Among different reagents, chlorosulfonic acid along with thionyl chloride in chloroform was obtained in good yield (Table 2: entry 8, 90%). The DCM solvent takes a long time to consume complete starting materials and gives fewer yields when compared with chloroform (Table 2: entry 6, 78%).

Among TEA and DIPEA, DIPEA in DCM was obtained in good yield (Table 3: entry 8, 55%). The THF solvent takes a long time (24 h) for the completion of the starting materials (Table 3: entry 10, 47%). In TEA, the reaction yields are less

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Table 1. Methylation on 3,5-Dimethyl-1*H*-pyrazole in Different Conditions[a]

s. no.	base	solvent	time (h)	yield (%)
1	K_2CO_3	THF	36	no reaction
2	K_2CO_3	DMF	32	15
3	K_2CO_3	CH_3CN	40	15
4	K_2CO_3	acetone	48	no reaction
5	Na_2CO_3	THF	34	no reaction
6	Na_2CO_3	DMF	32	18
7	NaOH	CH_3CN	32	20
8	NaOH	acetone	36	no reaction
9	NaOH	THF	26	27
10	NaOH	DMF	24	32
11	NaH	THF	16	48
12	NaH	DMF	12	55
13	potassium *tert*-butoxide	THF	16	78

[a]All reactions were carried out on a 100 mg scale. The progress of the reaction was monitored by TLC and LC−MS. Amine (1.0 equiv), methyl iodide (1.3 equiv), base (1.8 equiv), and solvent (10 vol).

Table 2. Sulfonylation on 3,5-Dimethyl-1*H*-pyrazole and 1,3,5-Trimethyl-1*H*-pyrazole in Different Conditions[a]

s. no.	sulfonyl chlorinating reagent	solvent	time (h)	temperature (°C)	yield (%)
1	chlorosulfonic acid	DCM	36	25−30	40
2	chlorosulfonic acid	chloroform	24	25−30	48
3	chlorosulfonic acid	DCM	24	40−50	50
4	chlorosulfonic acid	chloroform	24	40−50	60
5	chlorosulfonic acid, thionyl chloride	DCM	24	25−30	45
6	chlorosulfonic acid, thionyl chloride	DCM	24	50	78
7	chlorosulfonic acid, thionyl chloride	chloroform	24	25−30	55
8	chlorosulfonic acid, thionyl chloride	chloroform	12	60	90

[a]All reactions were carried out on a 100 mg scale. The progress of the reaction was monitored by TLC and LC−MS. Pyrazole compound (1.0 equiv), chlorosulfonic acid (5.5 equiv), thionyl chloride (1.32 equiv), and solvent (10 vol).

Table 3. Sulfonamide Preparation by Using Different Bases and Solvents[a]

s. no.	base	solvent	temperature (°C)	time (h)	yield (%)
1	TEA	DCM	0−10	24	26
2	TEA	DCM	RT	14	32
3	TEA	chloroform	0−10	24	35
4	TEA	chloroform	RT	16	46
5	TEA	THF	0−10	16	46
6	TEA	THF	RT	16	46
7	DIPEA	DCM	0−10	28	30
8	DIPEA	DCM	RT	16	55
9	DIPEA	THF	0−10	24	22
10	DIPEA	THF	RT	24	47

[a]All reactions were carried out on a 100 mg scale. The progress of the reaction was monitored by TLC and LC−MS. Sulfonyl chloride compound (1.0 equiv), amine (1.05 equiv), base (1.5 equiv), and solvent (10 vol).

when compared to DIPEA (Table 3: entry 1−6, 26−46%). DIPEA is a better base when compared to Et_3N.

After optimizing the reaction conditions for sulfonamide coupling with DIPEA in DCM for 16 h, we prepared the remaining 18 compounds by using the optimized standard condition.

Among the nineteen 3,5-dimethyl-*N*-phenethyl-1*H*-pyrazole-4-sulfonamide and 1,3,5-trimethyl-*N*-phenethyl-1*H*-pyrazole-4-sulfonamide derivatives (MR-S1-1 to MR-S1-19), compound MR-S1-13 gave the best yield (Table 4: entry 13, 71%) and compound MR-S1-5 gave low yields (Table 4: entry 5, 41%).

Cancer has been known for hyperproliferative disorders of cells, being one of the leading causes of death throughout the world, and presently, the main therapeutic options involve surgery, radiotherapy, and chemotherapy. There has always been a need for novel compounds with potential anticancer activity. From ages of cancer therapeutics and enhanced biological knowledge, novel synthetic chemistry has had a central role in the anticancer agent discovery. Although several mechanisms of cancer cell proliferation have been established, the effective anticancer drug is still under investigation. Various chemical compound series have been evaluated but there is still a need to find compounds with anticancer properties, with related mechanism as metastasis, cancer cell chemoresistance.

Previously, a series of new pyrazole sulfonamide derivatives were reported elsewhere for their anticancer activity (Samet et al.,[17]). Most of the tested derivatives showed moderate to excellent activity against the tested-on rat brain tumor cell lines (C6).

There has always been a need to get novel series of compounds, which exhibits good antiproliferative or cytotoxic compounds that displayed distinct antiproliferative properties.[16−20] Exploring small molecule antitumor compounds, which could decrease drug resistance and reduce unnecessary side effects, is more desirable. These compounds are further tested to know any unwanted cytotoxic activity on these cancer cells, and the results showed that they were not cytotoxic, which might be safe.

Structural Elucidation of *N*-(2-(Cyclohex-1-en-1-yl)-ethyl)-1,3,5-trimethyl-1*H*-pyrazole-4-sulfonamide (MR-S1-2) by FT-IR, ^{1}H NMR, ^{13}C NMR, and HRMS Spectra. IR (KBr, cm^{-1}): 3284 (NH), 2934 (asymmetric and symmetric stretching of CH_3), 1636 (C═C), 1530 (C═N), 1414, 1316 (asymmetric and symmetric deformation of CH_3), 1209 (CH_2), 1145 (SO_2), 1104 (C−N), 1062 (N−N), 691 (C-CH_3) (Figure 2). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 5.43 (s, 1H, NH), 4.28 (t, j = 6 Hz, 1H, C═C−H), 3.74 (s, 3H, N-CH_3), 2.96 (q, j = 6.5 Hz, 7.2 Hz, 2H, N-CH_2), 2.46 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.1 (t, j = 6.6 Hz, 2H, CH_2), 1.98 (t, j = 3.5 Hz, 2H, CH_2), 1.69 (t, j = 3.6 Hz, 2H, CH_2), 1.54(m, 4H, $2CH_2$) (Figure 3). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 145.64 (Ar-C), 141.12 (Ar-C), 134.37 (C═C), 122.14 (C═C), 115.64 (Ar-C), 40.54 (N-CH_2), 37.2 (CH_3), 36.0 (CH_2), 27.5 (CH_2), 24.63 (CH_2), 22.31 (CH_2), 21.83 (CH_2), 12.76 (CH_3), 10.06 (CH_3) (Figure 4). HRMS (ESI) m/z calcd for $[C_{14}H_{23}N_3O_2S]^+$: 297.42 $[M + H]^+$, found 298.3 (Figure 5).

EXPERIMENTAL SECTION

General Procedures and Instrumentation. FT-IR spectra were recorded on Jasco instrument with model FT/IR-4100 type A by using spectra manager software by taking 3−5 mg of compound on 250−300 mg of KBr salt. ^{1}H NMR spectra were obtained using 400 and 500 MHz spectrometer with $CDCl_3$ and DMSO-d_6 as solvents. Chemical shifts are

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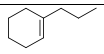
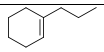
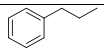
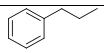
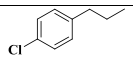
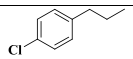
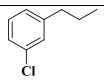
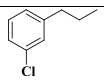
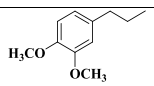
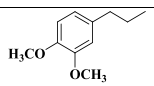
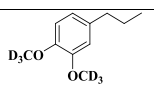
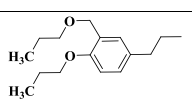
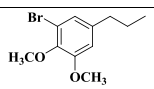
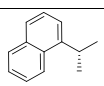
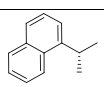
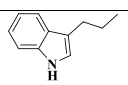
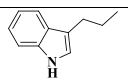
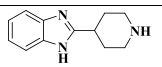
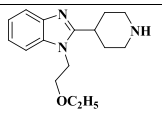
Table 4. Synthesis of 3,5-Dimethyl-*N*-phenethyl-1*H*-pyrazole-4-sulfonamide and 1,3,5-Trimethyl-*N*-phenethyl-1*H*-pyrazole-4-sulfonamide[a]

s. no.	compound	R	R^1	yield
1	MR-S1-1		H	65
2	MR-S1-2		CH_3	67
3	MR-S1-3		H	52
4	MR-S1-4		CH_3	55
5	MR-S1-5		H	41
6	MR-S1-6		CH_3	49
7	MR-S1-7		H	54
8	MR-S1-8		CH_3	51
9	MR-S1-9		H	64
10	MR-S1-10		CH_3	60
11	MR-S1-11		H	58
12	MR-S1-12		H	62
13	MR-S1-13		H	71
14	MR-S1-14		H	58
15	MR-S1-15		CH_3	53
16	MR-S1-16		H	55
17	MR-S1-17		CH_3	49
18	MR-S1-18		H	51
19	MR-S1-19		H	42

[a]All reactions were carried out on a 100 mg scale. The progress of the reaction was monitored by TLC and LC−MS. Sulfonyl chloride (1.0 equiv), amine (1.05 equiv), DIPEA (3.0 equiv), and DCM (10 vol).

calculated in parts per million relative to the residual solvent resonance for ^{1}H and ^{13}C NMR ($CDCl_3$ = 7.26 ppm for ^{1}H and 77.2 ppm for ^{13}C, DMSO-d_6 = 2.5 ppm for ^{1}H, 39.2 ppm for ^{13}C downfield from tetramethyl silane as the internal standard. Coupling constants (J values) are reported in Hertz (Hz). High-resolution mass spectrometry (HRMS) spectra were obtained by using the ESI technique, positive mode, capillary 4500, 0.4 Bar, dry gas 4.0 L min^{-1}. Analytical TLC analysis was performed by using silica-gel plates (Merck DC Silica plates 60 GF254). Visualization of compounds was done by illumination with 254 nm UV lamp. Flash column chromatography was performed on Merck silica gel (mesh size, 100−200) using the indicated solvents.

All reagents and solvents were used directly from the manufacturer and purified when required by standard procedures.

3,5-Dimethyl-1H-pyrazole. White solid. Yield 32 g, 95%, ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 5.78 (s, 1H), 5.29 (s, 1H), 2.4 (s, 6H).

1,3,5-Trimethyl-1H-pyrazole. Brown solid. Yield 27 g, 78%, ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 5.78 (s, 1H), 3.69 (s, 3H), 2.21 (t, j = 2.4 Hz, 6H).

3,5-Dimethyl-1H-pyrazole-4-sulfonyl Chloride. Pale yellow solid. Yield 25 g, 90%, ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 15.16 (s, 1H), 2.41 (s, 3H), 2.4 (s, 3H).

1,3,5-Trimethyl-1H-pyrazole-4-sulfonyl Chloride. Pale yellow solid. Yield 25 g, 90%. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 3.79 (s, 3H), 2.55 (s, 3H), 2.47 (s, 3H).

N-(2-(Cyclohex-1-en-1-yl)ethyl)-3,5-dimethyl-1H-pyrazole-4-sulfonamide (MR-S1-1). 95 mg, 65% yield, IR (KBr, cm^{-1}): 3291, 2938, 2362, 1668, 1567, 1423, 1316, 1185, 1113, 1075, 681. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 11−10.1 (b, 1H), 5.43 (s, 1H), 4.39 (t, j = 6.5 Hz, 1H), 2.97 (q, j = 6 Hz, 6.5 Hz, 2H), 2.45 (s, 6H), 2.11 (t, j = 6.5 Hz, 2H), 1.98 (t, j = 2 Hz, 2H), 1.77 (t, j = 3.5 Hz, 2H), 1.55 (m, 4H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 147.13, 141.63, 134.39, 122.18, 115.17, 40.56, 37.2, 27.5, 24.65, 22.33, 21.85, 12.95, 10.43. HRMS (ESI) m/z calcd for $[C_{13}H_{21}N_3O_2S]^+$: 283.39 $[M + H]^+$, found 284.4.

3,5-Dimethyl-N-phenethyl-1H-pyrazole-4-sulfonamide (MR-S1-3). 75 mg, 52% yield, IR (KBr, cm^{-1}): 3292, 3156, 3081, 2952, 2863, 2362, 1567, 1429, 1311, 1186, 1114, 1031, 941, 907, 838, 744, 660. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 7.26 (m, 3H), 7.09 (t, j = 7 Hz, 2H), 4.30 (t, j = 6 Hz, 1H), 3.21 (q, j = 6.5 Hz, 6.5 Hz, 2H), 2.81 (t, j = 6.5 Hz, 3H), 2.35 (s, 6H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 144.51, 138.94, 128.57, 128.3, 126.17, 114.89, 43.48, 35.06, 11.70. HRMS (ESI) m/z calcd for $[C_{13}H_{17}N_3O_2S]^+$: 279.36 $[M + H]^+$, found 280.1.

1,3,5-Trimethyl-N-phenethyl-1H-pyrazole-4-sulfonamide (MR-S1-4). 77 mg, 55% yield, IR (KBr, cm^{-1}): 3399, 2928, 2362, 1636, 1523, 1456, 1418, 1313, 1207, 1148, 1107, 1080, 817, 743, 681. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 7.27 (m, 3H), 7.1 (dd, j = 2.1 Hz, 1.5 Hz, 2H), 4.28 (t, j = 6 Hz, 3H), 3.71 (s, 3H), 3.18 (q, j = 6.9 Hz, 6.6 Hz, 3H), 2.79 (t, j = 6.6 Hz, 3H), 2.35 (s, 3H), 2.27 (s, 3H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 145.67, 141.2, 138.9, 128.57, 128.42, 126.12, 115.38, 43.44, 36.01, 35.03, 12.8, 10.05. HRMS (ESI) m/z calcd for $[C_{14}H_{19}N_3O_2S]^+$: 293.38 $[M + H]^+$, found 294.2.

N-(4-Chlorophenethyl)-3,5-dimethyl-1H-pyrazole-4-sulfonamide (MR-S1-5). 66 mg, 41% yield, IR (KBr, cm^{-1}): 3294, 3162, 3084, 2936, 2860, 1637, 1567, 1493, 1418, 1320, 1187,

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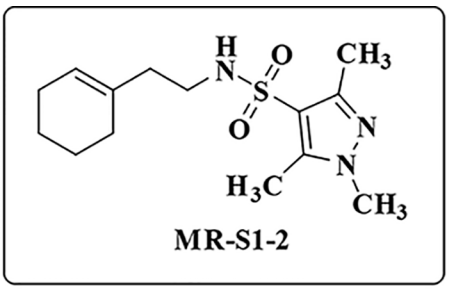

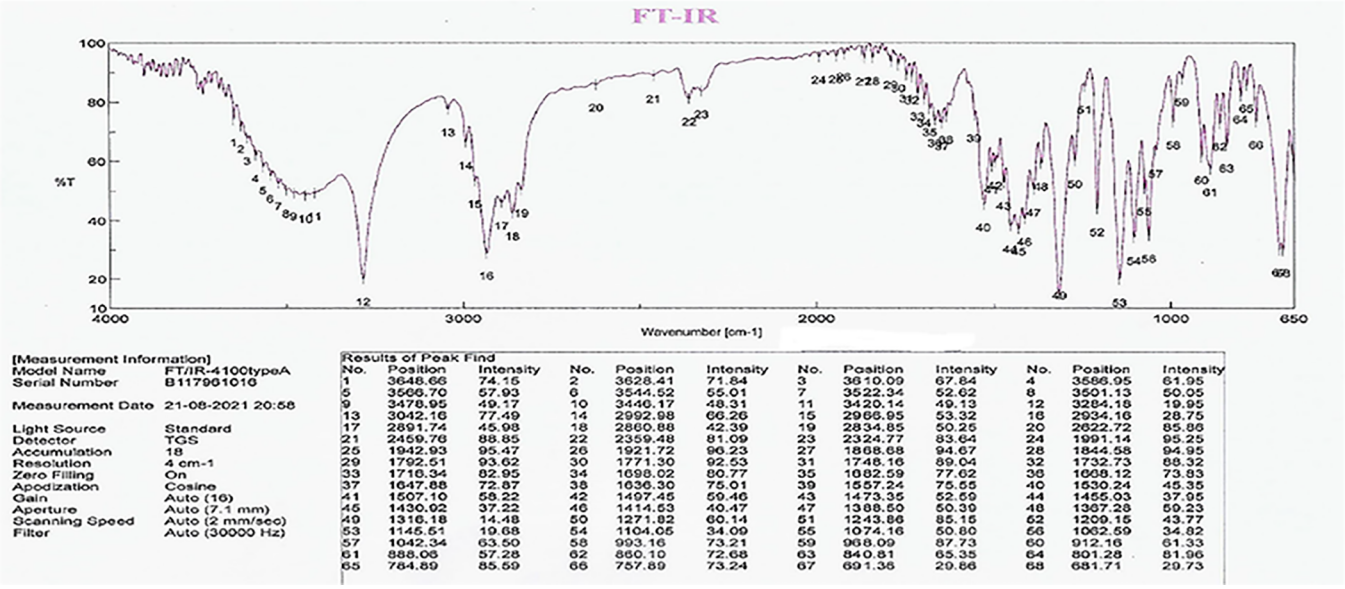

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3648.66	74.15	2	3628.41	71.84	3	3610.09	67.84	4	3586.95	61.95
5	3566.70	57.93	6	3544.52	55.01	7	3522.34	52.62	8	3501.13	50.05
9	3478.95	49.17	10	3446.17	48.31	11	3420.14	49.13	12	3284.18	19.95
13	3042.16	77.49	14	2992.98	66.26	15	2966.95	53.32	16	2934.16	28.75
17	2891.74	45.98	18	2860.88	42.39	19	2834.85	50.25	20	2622.72	95.86
21	2459.76	88.85	22	2359.48	81.09	23	2324.77	83.64	24	1991.14	95.25
25	1942.93	95.47	26	1921.72	96.23	27	1868.68	94.67	28	1844.58	94.95
29	1792.51	93.62	30	1771.30	92.53	31	1748.16	89.04	32	1732.73	88.32
33	1716.34	82.95	34	1698.02	80.77	35	1682.59	77.62	36	1668.12	73.83
37	1647.88	72.87	38	1636.30	75.01	39	1557.24	75.55	40	1530.24	45.35
41	1507.10	58.22	42	1497.46	59.46	43	1473.35	52.59	44	1455.03	37.05
45	1430.02	37.22	46	1414.53	40.47	47	1388.50	50.39	48	1367.28	59.23
49	1316.18	14.48	50	1271.82	60.14	51	1243.86	85.15	52	1209.15	43.77
53	1145.51	19.68	54	1104.05	34.09	55	1074.16	50.80	56	1062.59	34.82
57	1042.34	63.50	58	993.16	73.21	59	968.09	87.73	60	912.16	61.33
61	888.06	57.28	62	860.10	72.68	63	840.81	65.35	64	801.28	51.96
65	784.89	85.59	66	757.89	73.24	67	691.36	29.86	68	681.71	29.73

Figure 2. FT-IR spectra of *N*-(2-(cyclohex-1-en-1-yl)ethyl)-1,3,5-trimethyl-1*H*-pyrazole-4-sulfonamide (MR-S1-2) in $CDCl_3$.

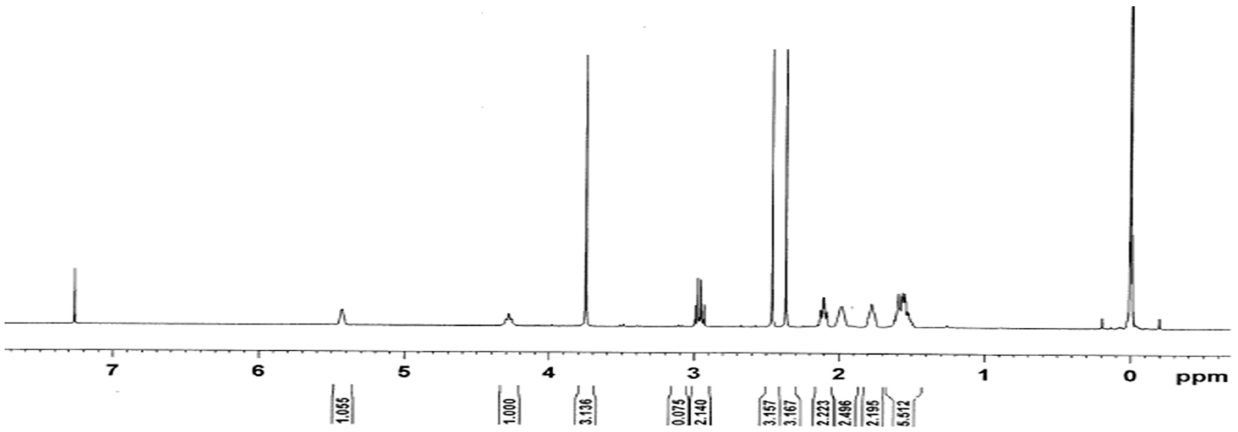

Figure 3. ^{1}H NMR spectra of *N*-(2-(cyclohex-1-en-1-yl)ethyl)-1,3,5-trimethyl-1*H*-pyrazole-4-sulfonamide (MR-S1-2) in $CDCl_3$.

1134, 1068, 1016, 935, 847, 814, 689. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 12.26 (b, 1H), 7.24 (dd, j = 1.5 Hz, 2.5 Hz, 2H), 7.11 (d, j = 8.5 Hz, 2H), 5.79 (t, j = 6 Hz, 2H), 3.09 (q, j = 6.5 Hz, 7.5 Hz, 2H), 2.76 (t, j = 7 Hz, 2H), 2.36 (s, 6H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 144.51, 138, 130.88, 130.5, 128.16, 114.88, 43.21, 34.3, 11.71. HRMS (ESI) m/z calcd for $[C_{13}H_{16}ClN_3O_2S]^+$: 313.8 $[M + H]^+$, found 314.3.

N-(4-Chlorophenethyl)-1,3,5-trimethyl-1H-pyrazole-4-sulfonamide (MR-S1-6). 77 mg, 49% yield, IR (KBr, cm^{-1}): 3157, 2935, 2859, 2362, 1647, 1523, 1492, 1409, 1328, 1208, 1150, 1079, 1013, 842, 804, 678. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 7.25 (dd, j = 2.1 Hz, 4.2 Hz, 2H), 7.04 (dd, j = 1.8 Hz, 6.6 Hz, 2H), 4.29 (t, j = 6.3 Hz, 1H), 3.72 (s, 3H), 3.17 (q, j = 6.6 Hz, 13.2 Hz, 2H), 2.77 (t, j = 6.9 Hz, 2H), 2.35 (s, 3H), 2.30 (s, 3H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 145.62, 141.11, 138, 130.8, 130.47, 128.07, 115.37, 43.16, 36, 34.22, 12.78, 10.04. HRMS (ESI) m/z calcd for $[C_{14}H_{18}ClN_3O_2S]^+$: 327.8 $[M + H]^+$, found 328.1.

N-(3-Chlorophenethyl)-3,5-dimethyl-1H-pyrazole-4-sulfonamide (MR-S1-7). 87 mg, 54% yield, IR (KBr, cm^{-1}): 3277, 3166, 3039, 2955, 2857, 2364, 2364, 1564, 1418, 1317, 1185, 1114, 1056, 938, 772, 691. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 10.1 (b, 1H), 7.24 (d, j = 2 Hz, 2H), 7.01 (s, 1H), 6.99 (t, j = 4.5 Hz, 1H), 4.47 (t, j = 3 Hz, 1H), 3.21 (q, j = 6.5 Hz, 7

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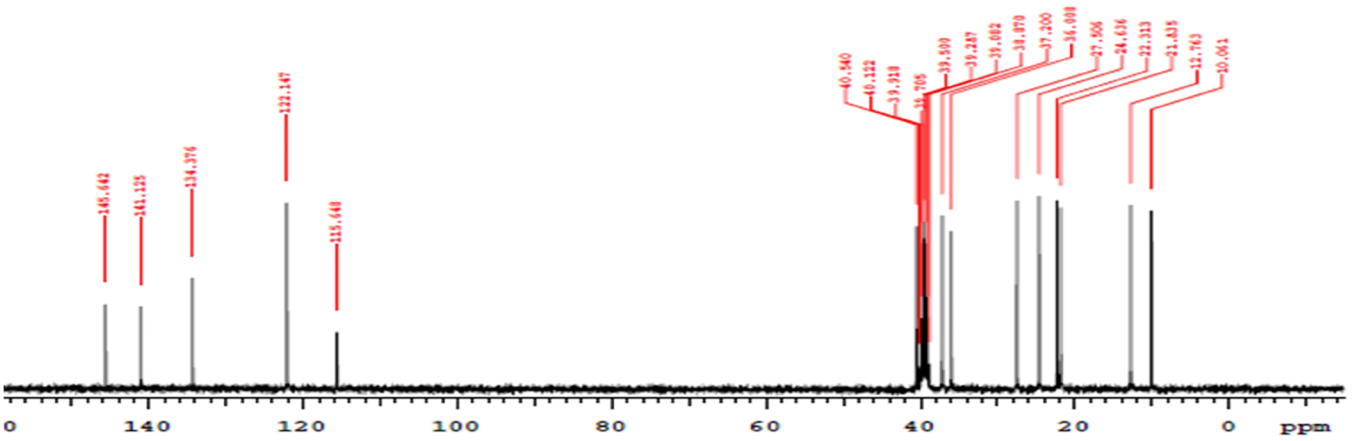

Figure 4. ^{13}C NMR spectra of *N*-(2-(cyclohex-1-en-1-yl)ethyl)-1,3,5-trimethyl-1*H*-pyrazole-4-sulfonamide (MR-S1-2) in $CDCl_3$.

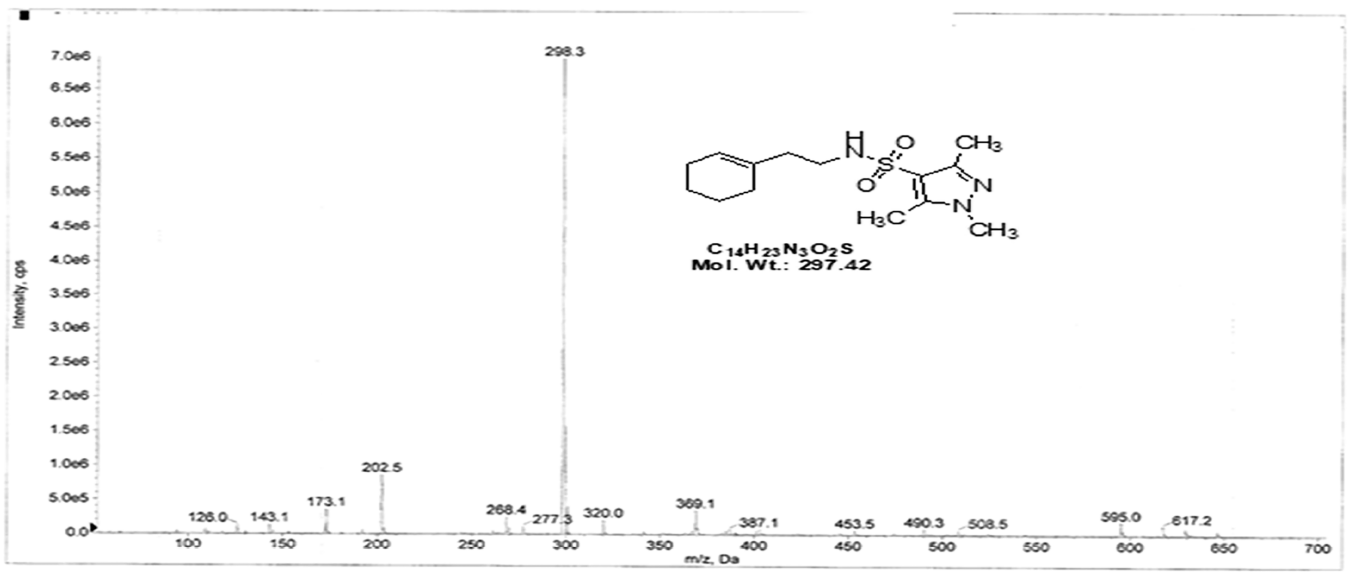

Figure 5. HRMS spectra of *N*-(2-(cyclohex-1-en-1-yl)ethyl)-1,3,5-trimethyl-1*H*-pyrazole-4-sulfonamide (MR-S1-2).

Hz, 2H), 2.78 (t, *j* = 6.5 Hz, 2H), 2.38 (s, 6H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 144.46, 141.63, 132.9, 130, 128.6, 128.3, 127.39, 126.15, 114.83, 43.03, 34.47, 11.7. HRMS (ESI) *m*/*z* calcd for $[C_{13}H_{16}ClN_3O_2S]^+$: 313.8 $[M + H]^+$, found 314.3.

N-(3-Chlorophenethyl)-1,3,5-trimethyl-1H-pyrazole-4-sulfonamide (MR-S1-8). 80 mg, 51% yield, IR (KBr, cm^{-1}): 3277, 3242, 2938, 2857, 2363, 1600, 1575, 1525, 1417, 1312, 1207, 1144, 1101, 936, 773, 691. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 7.25 (dd, *j* = 2.1 Hz, 1.2 Hz, 2H), 7.06 (d, *j* = 1.5 Hz, 1H), 7.02 (t, *j* = 1.5 Hz, 1H), 4.35(b, 1H), 3.73 (s, 3H), 3.19 (q, *j* = 3.6 Hz, 1.8 Hz, 2H), 2.77 (t, *j* = 6.6 Hz, 2H), 2.37 (s, 3H), 2.30 (s, 3H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 145.64, 141.6, 141.12, 132.81, 129.94, 128.54, 127.37, 126.09, 115.34, 43, 36, 34.43, 12.77, 10.03. HRMS (ESI) *m*/*z* calcd for $[C_{14}H_{18}ClN_3O_2S]^+$: 327.8 $[M + H]^+$, found 328.1.

N-(3,4-Dimethoxyphenethyl)-3,5-dimethyl-1H-pyrazole-4-sulfonamide (MR-S1-9). 112 mg, 64% yield, IR (KBr, cm^{-1}): 3276, 3113, 2914, 2836, 1594, 1562, 1519, 1423, 1239, 1186, 1139, 1114, 1022, 943, 800, 690. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 6.79 (d, *j* = 8.1 Hz, 1H), 6.65 (dd, *j* = 1.8 Hz, 5.1 Hz, 1H), 6.30 (d, *j* = 1.8 Hz, 1H), 4.37 (t, *j* = 5.7 Hz, 1H), 3.84 (d, *j* = 6 Hz, 6H), 3.18 (q, *j* = 6.6 Hz, 6.3 Hz, 2H), 2.75 (t, *j* = 6.6 Hz, 2H), 2.35 (s, 6H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 148.62, 147.32, 147.2, 141.76, 131.37, 120.44, 115, 112.45, 111.88, 55.51, 55.37, 43.7, 34.69, 13, 10.45. HRMS (ESI) *m*/*z* calcd for $[C_{15}H_{21}N_3O4S]^+$: 339.41 $[M + H]^+$, found 340.3.

N-(3,4-Bis(methoxy-d3)phenethyl-d6)-3,5-dimethyl-1H-pyrazole-4-sulfonamide (MR-S1-10). 103 mg, 60% yield, IR (KBr, cm^{-1}): 3247, 2966, 2942, 2834, 2363, 1594, 1517, 1445, 1318, 1264, 1233, 1148, 1082, 1033, 940, 844, 820, 763, 701, 681. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 6.78 (d, *j* = 8.1 Hz, 1H), 6.65 (dd, *j* = 1.8 Hz, 6.3 Hz, 1H), 6.60 (d, *j* = 2.1 Hz, 1H), 4.28 (t, *j* = 6.3 Hz, 1H), 3.85 (d, *j* = 5.1 Hz, 6H), 3.71 (s, 3H), 3.16 (q, *j* = 6.6 Hz, 6.3 Hz, 2H), 2.74 (t, *j* = 6.6 Hz, 2H), 2.34 (s, 3H), 2.29 (s, 3H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 148.55, 147.26, 145.65, 141.11, 131.32, 120.45, 115.45, 112.37, 111.78, 55.47, 55.31, 43.64, 35.95, 34.63, 12.81, 10.03. HRMS (ESI) *m*/*z* calcd for $[C_{16}H_{23}N_3O_4S]^+$: 353.44 $[M + H]^+$, found 353.9.

N-(3,4-Dimethoxyphenethyl)-1,3,5-trimethyl-1H-pyrazole-4-sulfonamide (MR-S1-11). 102 mg, 58% yield, IR (KBr, cm^{-1}): 3293, 3113, 2971, 2937, 2860, 2362, 2218, 2069, 1614, 1561, 1516, 1419, 1315, 1275, 1243, 1186, 1114, 939, 800, 690, 675. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 6.78 (d, *j* =

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8.5 Hz, 1H), 6.65 (dd, j = 6.5 Hz, 6 Hz, 1H), 6.59 (s, 1H), 4.51 (t, j = 6.5 Hz, 1H), 3.18 (q, j = 6.5 Hz, 13 Hz, 2H), 2.75 (t, j = 4.2 Hz, 2H), 2.34 (s, 6H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 148.61, 147.3, 144.5, 131.62, 120.41, 114.98, 112.44, 111.87, 43.73, 34.69, 11.74. HRMS (ESI) m/z calcd for $[C_{15}H_{15}D_6N_3O_4S]^+$: 345.45 $[M + H]^+$, found 346.2.

N-(3,4-Dipropoxyphenethyl)-3,5-dimethyl-1H-pyrazole-4-sulfonamide (MR-S1-12). 126 mg, 62% yield, IR (KBr, cm^{-1}): 3338, 3173, 3100, 2963, 2934, 2874, 2364, 1589, 1519, 1410, 1318, 1231, 1185, 1139, 1114, 1047, 979, 882, 862, 804, 653. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 6.79 (d, j = 9 Hz, 2H), 6.61 (dd, j = 2.5 Hz, 2 Hz, 2H), 3.92 (q, j = 6.5 Hz, 7 Hz, 4H), 3.16 (t, j = 6.5 Hz, 2H), 2.72 (q, j = 4 Hz, 6.5 Hz, 2H), 2.50 (s, 6H), 1.80 (m, 4H), 1.11 (q, j = 7.5 Hz, 7 Hz, 6H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 148.5, 147.04, 131.63, 120.69, 120.6, 114.94, 114.52, 114.13, 70.05, 69.82, 43.69, 34.62, 34.51, 22.23, 11.69, 10.41. HRMS (ESI) m/z calcd for $[C_{19}H_{29}N_3O_4S]^+$: 395.52 $[M + H]^+$, found 396.5.

N-(3-Bromo-4,5-dimethoxyphenethyl)-3,5-dimethyl-1H-pyrazole-4-sulfonamide (MR-S1-13). 153 mg, 71% yield, IR (KBr, cm^{-1}): 3325, 3290, 2971, 2932, 2846, 11,646, 1557, 1509, 1420, 1384, 1319, 1260, 1184, 1114, 1038, 929, 858, 746, 681. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 6.98 (d, j = 10.2 Hz, 2H), 6.68 (d, j = 11.7 Hz, 2H), 4.48 (t, j = 6 Hz, 1H), 3.75 (s, 2H), 3.20 (q, j = 6.9 Hz, 6.6 Hz, 2H), 2.89 (t, j = 3 Hz, 2H), 2.39 (s, 6H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 169.2, 148.17, 144.48, 129.64, 115.52, 114.96, 114.17, 113.23, 55.85, 55.62, 41.86, 35.1, 22.61, 11.74. HRMS (ESI) m/z calcd for $[C_{15}H_{20}BrN_3O_4S]^+$: 418.31 $[M + H]^-$, found 417.7.

(R)-3,5-Dimethyl-N-(1-(naphthalen-1-yl)ethyl)-1H-pyrazole-4-sulfonamide (MR-S1-14). 98 mg, 58% yield, IR (KBr, cm^{-1}): 3292, 3100, 2975, 2932, 2869, 2364, 1560, 1418, 1374, 1307, 1187, 1111, 95, 872, 800, 778, 689. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 7.97 (t, j = 3 Hz, 1H), 7.83 (t, j = 2.4 Hz, 1H), 7.70 (dd, j = 6 Hz, 4.5 Hz, 1H), 7.49 (m, 2H), 7.34 (d, j = 1.8 Hz, 2H), 5.32 (m, 1H), 4.95 (d, j = 7.2 Hz, 1H), 2.20 (s, 6H), 1.61 (t, j = 6.9 Hz, 3H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 146.82, 141.37, 139.24, 133.22, 129.62, 128.67, 127.15, 126.1, 125.45, 125.2, 123.12, 122.51, 116, 48.52, 23.3, 12.98, 10.26. HRMS (ESI) m/z calcd for $[C_{17}H_{19}N_3O_2S]^+$: 329.42 $[M + H]^+$, found 330.3.

(R)-1,3,5-Trimethyl-N-(1-(naphthalen-1-yl)ethyl)-1H-pyrazole-4-sulfonamide (MR-S1-15). 87 mg, 53% yield, IR (KBr, cm^{-1}): 3446, 3294, 3048, 2976, 2930, 2364, 1539, 1449, 1323, 1298, 1208, 1151, 1107, 1076, 953, 867, 779, 688. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 7.81 (t, j = 3.3 Hz, 1H), 7.72 (m, 1H), 7.70 (m, 1H), 7.49 (m, 2H), 7.32 (d, j = 4.5 Hz, 2H), 5.29 (m, 1H), 4.89 (d, j = 5.7 Hz, 1H), 3.40 (s, 3H), 2.23 (s, 3H), 1.95 (s, 3H), 1.64 (d, j = 6.9 Hz, 3H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 145.31, 140.73, 139.1, 133.1, 129.59, 128.57, 126.99, 125.99, 125.4, 125, 123.16, 122.5, 116.35, 48.53, 35.55, 23.34, 12.69, 9.82. HRMS (ESI) m/z calcd for $[C_{18}H_{21}N_3O_2S]^+$: 343.44 $[M + H]^+$, found 344.5.

N-(2-(1H-Indol-3-yl)ethyl)-3,5-dimethyl-1H-pyrazole-4-sulfonamide (MR-S1-16). 90 mg, 55% yield, IR (KBr, cm^{-1}): 3425, 3331, 3053, 2945, 2845, 1624, 1563, 1457, 1423, 1348, 1282, 1186, 1115, 1047, 916, 813, 737. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 8.05 (b, 1H), 7.44 (d, j = 8 Hz, 1H), 7.37 (d, j = 8 Hz, 1H), 7.22 (t, j = 7 Hz, 1H), 7.10 (t, j = 1 Hz, 1H), 7.01 (d, j = 7.5 Hz, 1H), 4.35 (t, j = 6 Hz, 1H), 3.38 (q, j = 6.5 Hz, 7 Hz, 2H), 2.27 (s, 6H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 144.5, 136.23, 127.04, 122.86, 121, 118.34, 117.96, 115.19, 111.47, 111.22, 42.98, 25.3, 11.77. HRMS (ESI) m/z calcd for $[C_{15}H_{18}N_4O_2S]^+$: 318.39 $[M + H]^+$, found 318.9.

N-(2-(1H-Indol-3-yl)ethyl)-1,3,5-trimethyl-1H-pyrazole-4-sulfonamide (MR-S1-17). 78 mg, 49% yield, IR (KBr, cm^{-1}): 3388, 3340, 3302, 3243, 3054, 2938, 2363, 1646, 1536, 1455, 1396, 1315, 1209, 1144, 1105, 999, 918, 826, 749, 682. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 8.01 (b, 1H), 7.42 (d, j = 8 Hz, 1H), 7.37 (d, j = 7 Hz, 1H), 7.22 (t, j = 7 Hz, 1H), 7.10 (t, j = 7.5 Hz, 1H), 7.06 (d, j = 2.5 Hz, 1H), 4.30 (t, j = 6 Hz, 1H), 3.62 (s, 3H), 3.25 (q, j = 6.5 Hz, 2H), 2.97 (t, j = 6.5 Hz, 2H), 2.24 (s, 3H), 2.17 (s, 3H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 145.65, 141.13, 136.2, 126.99, 122.9, 120.94, 118.27, 117.93, 115.58, 111.41, 111.14, 42.86, 35.99, 25.24, 12.83, 10.03. HRMS (ESI) m/z calcd for $[C_{16}H_{20}N_4O_2S]^+$: 332.42 $[M + H]^+$, found 374.2.

2-(1-((1,3,5-Trimethyl-1H-pyrazol-4-yl)sulfonyl)piperidin-4-yl)-1H-benzo[d]imidazole (MR-S1-18). 92 mg, 51% yield, IR (KBr, cm^{-1}): 3446, 3181, 2950, 2925, 2362, 1636, 1528, 1455, 1428, 1329, 1272, 1149, 1106, 1052, 992, 933, 750, 720, 683. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm): 7.53 (b, 2H), 7.19 (dd, j = 4 Hz, 3.5 Hz, 2H), 3.78 (m, 4H), 2.95 (m, 1H), 2.63 (t, j = 11.5 Hz, 2H), 2.47 (s, 3H), 2.39 (s, 3H), 2.20 (m, 2H), 2.10 (t, j = 3.5 Hz, 2H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 156.91, 146.42, 142.27, 121.27, 114.55, 111.1, 45.01, 36.31, 34.69, 29.6, 13.22, 10.58. HRMS (ESI) m/z calcd for $[C_{18}H_{23}N_5O_2S]^+$: 373.47 $[M + H]^+$, found 374.2.

2-(1-((3,5-Dimethyl-1H-pyrazol-4-yl)sulfonyl)piperidin-4-yl)-1-(2-ethoxyethyl)-1H-benzo[d]imidazole (MR-S1-19). 93 mg, 42% yield, IR (KBr, cm^{-1}): 3445, 3065, 2929, 2858, 2362, 1646, 1507, 1467, 1437, 132, 1187, 1139, 1121, 927, 748, 729. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 7.71 (m, 1H), 7.29 (m, 3H), 4.30 (t, j = 5.1 Hz, 2H), 3.86 (d, j = 5.1 Hz, 2H), 3.70 (t, j = 5.1 Hz, 2H), 3.36 (q, j = 7.2 Hz, 6.9 Hz, 2H), 3.07 (m, 1H), 2.74 (t, j = 3.3 Hz, 2H), 2.48 (s, 6H), 2.12 (m, 4H), 1.07 (t, j = 7.2 Hz, 3H). ^{13}C NMR spectrum, δ_c, ppm, DMSO-d_6: 157.68, 147.94, 142.8, 142.24, 134.77, 121.54, 121.29, 118.5, 110.54, 110.32, 68.47, 65.74, 45.36, 42.95, 32.16, 30.28, 14.82, 13.42, 10.82. HRMS (ESI) m/z calcd for $[C_{21}H_{29}N_5O_3S]^+$: 431.55 $[M + H]^+$, found 431.9.

Anticancer Activity Assessment. The in vitro antiproliferative activity was estimated for the compound named MR-S1-6 against U937 cells by the CellTiter-Glo Luminescent cell viability assay using Mitomycin C as a positive control. Briefly, the live cells were incubated with various concentrations of compounds for 72 h. To the cell culture medium, the CellTiter-Glo reagent was added equal to the volume. These contents are further mixed on an orbital shaker for complete cell lysis for 2 min. At room temperature, these plates were allowed to incubate for 10 min for luminescent signal stabilization. Luminescence signals were captured by using the luminescence module under a BMG Fluostar Omega microplate reader. Viability data are calculated using the following formula: (RLU) of test cells × 100/(RLU) of control cells. Furthermore, this cell viability was calculated for inhibitory activity of compounds for these cells. The half maximal inhibitory concentration (IC_{50}) was calculated by Graph Pad Prism software for each dose.

Cytotoxic Activity Evaluation. Many therapeutic agents need multiple reproduction cycles for the killing effect to be evident, and dosage-dependent direct cell killing can act which can be confirmed using the LDH-based cytotoxicity assay. Cytotoxicity detection is based on the measurement of LDH activity released from damaged cells, where plasma-membrane

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Table 5. LDH Release Assay Determines the Cytotoxicity Activity, Optical Density at 450 nm, and Present Activity[a]

	OD at 450 nm at various concentrations of compounds				percent inhibition at various concentrations of compounds			
compound ID	100 μM	50 μM	25 μM	12.5 μM	100 μM	50 μM	25 μM	12.5 μM
MR-S1-1	0.113	0.119	0.111	0.117	−1.8	−0.4	−2.2	−0.9
MR-S1-2	0.097	0.107	0.105	0.115	−5.3	−3.1	−3.6	−1.3
MR-S1--3	0.115	0.115	0.11	0.117	−1.3	−1.3	−2.4	−0.9
MR-S1-4	0.113	0.117	0.111	0.108	−1.8	−0.9	−2.2	−2.9
MR-S1-5	0.109	0.12	0.111	0.113	−2.7	−0.2	−2.2	−1.8
MR-S1-6	0.114	0.112	0.115	0.136	−1.6	−2.0	−1.3	3.3
MR-S1-7	0.107	0.125	0.11	0.112	−3.1	0.9	−2.4	−2.0
MR-S1-8	0.123	0.129	0.115	0.134	0.4	1.8	−1.3	2.9
MR-S1-9	0.11	0.132	0.133	0.109	−2.4	2.4	2.7	−2.7
MR-S1-10	0.121	0.112	0.139	0.107	0.0	−2.0	4.0	−3.1
MR-S1-11	0.138	0.117	0.132	0.11	3.8	−0.9	2.4	−2.4
MR-S1-12	0.117	0.109	0.124	0.111	−0.9	−2.7	0.7	−2.2
MR-S1-13	0.122	0.117	0.136	0.118	0.2	−0.9	3.3	−0.7
MR-S1-14	0.107	0.117	0.103	0.11	−3.1	−0.9	−4.0	−2.4
MR-S1-15	0.109	0.113	0.115	0.121	−2.7	−1.8	−1.3	0.0
MR-S1-16	0.113	0.114	0.117	0.138	−1.8	−1.6	−0.9	3.8
MR-S1-17	0.114	0.112	0.112	0.117	−1.6	−2.0	−2.0	−0.9
MR-S1-18	0.119	0.122	0.113	0.122	−0.4	0.2	−1.8	0.2
MR-S1-19	0.103	0.113	0.115	0.099	−4.0	−1.8	−1.3	−4.9
positive control (Triton-X100, 0.1%)	0.595	0.587	0.545	0.557	100%			
negative control, DMSO	0.11	0.132	0.133	0.109	0%			

[a]For the cytotoxicity activity Triton-X100 was taken as reference positive control where DMSO as a diluent was considered as negative control where there is no cytotoxicity activity.

damage is determined. Cellular toxicity is measured using the absorbance changes of tetrazolium dyes which occur when enzymatically reduced by LDH released by damaged cells. LDH catalyzes the formation of lactate from pyruvate in the presence of NADH. The oxidation of NADH is proportional to LDH activity in the sample which increases the absorbance at 340 nm (Kendig and Tarloff[20]). Briefly, a clear, flat-bottom, 96-well plate was plated with U937 cells and incubated with different concentrations of compounds, and Triton X100 was taken as the positive cytotoxicity control. The plate was then incubated for four hours at 37 °C and centrifuged at 1000 RPM for 5 min. Supernatants were harvested to another 96-well clear, flat-bottom plate, and the LDH reagent (Cytotoxicity detection kit, Sigma) was added and incubated in the dark for 10 min at room temperature. The BMG Fluostar Omega microplate reader is used to measure the optical density at 490 nm. Further cytotoxicity activity of compounds for these cells was calculated by this optical density. By Graph Pad Prism software, the half maximal inhibitory concentration (IC_{50}) was calculated for each dose.

RESULTS AND DISCUSSION

In this paper, we defined the chemistry for the preparation of pyrazole-4-sulfonamide derivatives. Pentane-2,4-dione with an active methylene group is treated with hydrazine hydrate in methanol. Dehydration and subsequent cyclization result in the formation of 3,5-dimethyl-1*H*-pyrazole with 95% yield. The reaction of 3,5-dimethyl-1*H*-pyrazole with chlorosulfonic acid and thionyl chloride leads to the formation of chlorosulfonyl pyrazole intermediate with appreciable yield.

Optimization studies were done for sulfonylation and methylation reaction using different bases, and various solvents and temperatures were used for achieving good yields and lower reaction times.

Methylation on 3,5-dimethyl-1*H*-pyrazole was studied using different solvents, bases, and temperatures to obtain higher yields. When methylation was done using potassium carbonate as a base and THF or acetone as a solvent, there was no product formation (Table 1, entry 1, 4), whereas the use of DMF or acetonitrile as a solvent resulted in the methylated product with very low yield (Table 1, entry 2, 3). There was no product formation when sodium carbonate is used as a base and THF as a solvent (Table 1, entry 5) but 18% of yield was obtained when DMF was used as a solvent (Table 1, entry 6). The product yield was more when sodium hydroxide or sodium hydride was used as a base (Table 1, entries 10, 12).

We have attained the maximum yield (78%) in methylation, when potassium tertiary butoxide was used as the base and THF as a solvent (Table 1, entry 13).

When sulfonylation was attempted with chlorosulfonic acid using different solvents, it resulted in sulfonyl chloride intermediate with a moderate yield (Table 2, entry 4). We have observed that the sulfonyl chloride intermediate degrades to sulfonic acid. To avoid the formation of sulfonic acid, a mixture of chlorosulfonic acid and thionyl chloride was used as the sulfonylating agent and the reaction was performed at 60 °C. Here, we have attained 90% yield in 12 h (Table 2, entry 8).

These 1,3,5-trimethyl-1*H*-pyrazoles were also reacted with chlorosulfonic acid and thionyl chloride to generate the chlorosulfonyl group on pyrazole.

The chlorosulfonyl intermediate is treated with different ethyl amines to give the final pyrazole sulfonamide derivatives in the presence of the Hunigs base. The structures of these derivatives were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy (MS).

Biological Activity Assessment. Although numerous anticancer drugs have been prepared in the past for

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Table 6. CellTiter-Glo Assay Determines the Antiproliferative Activity RLU Count and Percent Inhibition[a]

	RLU at various concentrations of compounds				percent inhibition at various concentrations of compounds			
compound ID	200 μM	100 μM	50 μM	25 μM	200 μM	100 μM	50 μM	25 μM
MR-S1-1	923	1146	1589	1943	64.2	54.2	34.5	18.6
MR-S1-2	1061	1415	1705	1806	58.0	42.2	29.3	24.8
MR-S1-3	685	1649	1811	2077	74.8	31.8	24.5	12.7
MR-S1-4	1840	1515	1930	1621	23.2	37.8	19.2	33.0
MR-S1-5	599	983	1372	1280	78.7	61.5	44.2	48.3
MR-S1-6	1370	1725	1959	1453	44.2	28.4	17.9	40.5
MR-S1-7	1205	1819	2061	2037	51.6	24.2	13.4	14.5
MR-S1-8	1299	1587	1724	1799	47.4	34.6	28.4	25.1
MR-S1-9	2315	2836	2948	2371	2.0	−21.2	−26.2	−0.5
MR-S1-10	2684	3234	3199	2542	−14.5	−39.0	−37.5	−8.1
MR-S1-11	1649	2380	2360	1816	31.8	−0.9	0.0	24.3
MR-S1-12	137	110	236	1556	99.3	100.5	94.9	35.9
MR-S1-13	517	735	909	827	82.3	72.6	64.8	68.5
MR-S1-14	146	823	1625	1743	98.9	68.7	32.9	27.6
MR-S1-15	101	380	904	1924	100.9	88.5	65.1	19.5
MR-S1-16	1110	1856	1821	2036	55.9	22.5	24.1	14.5
MR-S1-17	2071	1639	2072	1943	12.9	32.2	12.9	18.6
MR-S1-18	494	1377	1546	2099	83.4	43.9	36.4	11.7
MR-S1-19	1958	2389	2311	2772	18.0	−1.3	2.2	−5.0
positive control (Mitomycin C, 10 μM)	142	143	99	103	100%			
negative control, DMSO	2170	2636	1985	2651	0%			

[a]For antiproliferative activity, Mitomycin C was taken as the reference positive control, and DMSO as the diluent of these compounds was considered as the negative control where there is no antiproliferative activity

chemotherapy, a desirable level of the therapeutic product has not yet been developed till now. In addition, these drugs have shown resistance against several cancer and their side effects, and there is still an unmet need for treating cancer. Still, efforts are underway to develop new anticancer drugs. In this present work, the newly synthesized 19 derivatives were tested for antiproliferative and cytotoxic activities, performed on the cell of human myeloid leukemia (U937). At a high concentration of 100 μM, none of the newly synthesized derivatives showed cytotoxicity in LDH release assay (Table 5) while these derivatives exhibited antiproliferative activities on U937 cells (Table 6). Active compounds in antiproliferative CellTiter-Glo assay were further calculated using Graph Pad Prism (Table 7) while these derivatives were not showing activity for cytotoxicity in LDH release assay.

Structure Activity Relationship. A series of new 19 pyrazole-4-sulfonamide derivatives with structural modifications at two different sites were synthesized, one on the sulfonamide site R (substituted cyclohexyl/phenyl ethyl amine compounds) and on the other site R_1 with the methyl group on the pyrazole ring. All the synthesized compounds were characterized, and they were screened for antiproliferative activity; these compounds were initially screened for cytotoxic activity, and from the cytotoxicity data, it is inferred that these compounds are not cytotoxic.

The antiproliferative activity (cytostatic activity) was measured using the CellTiter-Glo assay. The percentage of inhibition was measured in four different concentrations, and Mitomycin-C was used as a standard. From the cytostatic data, it is observed that five compounds (MR-S1-12, MR-S1-13, MR-S1-14, MR-S1-15, and MR-S1-18) are having good antiproliferative activity, three compounds showed moderate activity (MR-S1-1, MR-S1-3, and MR-S1-5), and four of them were impotent (MR-S1-4, MR-S1-9, MR-S1-17, and MR-S1-19).

Table 7. Graph Pad Prism Analysis Provides the Antiproliferative Activity (GI50) and Cytotoxicity Activity (LC50) by Using Percent Inhibition and Percent Activity, Respectively, of the Compounds

compound ID	antiproliferative (U937) GI50 (μM)	cytotoxicity LC50 (μM)
MR-S1-1	1.7	>100
MR-S1-2	>200	>100
MR-S1-3	16	>100
MR-S1-4	>200	>100
MR-S1-5	16	>100
MR-S1-6	>200	>100
MR-S1-7	>200	>100
MR-S1-8	>200	>100
MR-S1-9	>200	>100
MR-S1-10	>200	>100
MR-S1-11	>200	>100
MR-S1-12	3.3	>100
MR-S1-13	3.33	>100
MR-S1-14	9.4	>100
MR-S1-15	3	>100
MR-S1-16	>200	>100
MR-S1-17	>200	>100
MR-S1-18	92.61	>100
MR-S1-19	>200	>100

Replacing hydrogen on the pyrazole ring with the methyl group does not have any impact on the antiproliferative activity.

Compounds MR-S1-14 and MR-S1-15 emerged as the most potent compounds in the series. Both these compounds are having bicyclic aromatic rings substituted on the sulfonamide

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site; from this, it is proved that bulky hydrophobic areas are necessary for good anticancer activity.

Compound MR-S1-12 with di-propoxy groups on the phenyl ring is also found to be potent. Electron-donating groups on the phenyl ring will be beneficial for enhancing the anti-cancer activity.

Compound MR-S1-13 with di-methoxy and bromo groups also showed good activity, which confirmed that electron-donating groups on the phenyl group are essential for anti-cancer activity.

Compound has good activity; it is observed that the isatin group may have enhanced the anti-cancer activity.

Compounds MR-S1-1, MR-S1-3, and MR-S1-5 showed moderate anti-proliferative activity; all these three compounds do not have any substitution on cyclohexyl or phenyl rings. Hence, it is proved that cyclic substituted compounds are more active than unsubstituted compounds.

Compounds MR-S1-4, MR-S1-9, MR-S1-10, MR-S1-17, and MR-S1-19 showed less anti-proliferative activity. The common structural feature in these compounds is that few of them are MR-S1-4 which has a simple aromatic ring and MR-S1-9 and MR-S1-10 have methoxy substitutions on the aromatic ring; MR-S1-17 and MR-S1-19 are substituted with isatin.

From the structure activity relationship data, it is concluded that pyrazole-4-sulfonamide derivatives will serve as pharmacophores for anti-tumor compounds. These structures will become privileged scaffolds for anti-cancer activity.

CONCLUSIONS

In conclusion, we have synthesized two series of new nineteen Pyrazole-4-sulfonamide derivatives and screened them for Antiproliferative activity (GI50) and cytotoxicity activity (LC50). The present paper is of potential use for the synthesis of pyrazole-4-sulfonamide derivatives. Varieties of pyrazole-4-sulfonamide derivatives can be synthesized and can be screened for biological activities. The methods described in this paper are simple, decisive, and involve mild reaction conditions, and this will add an attractive procedure for the existing armory of pyrazole-4-sulfonamide derivative synthesis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c07539.

List of the contents of the materials supplied (PDF)

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Author Contributions

All the authors are responsible for the following: Study conception and design, data collection, analysis, interpretation of results and manuscript preparation. All the authors reviewed the results and approved the final manuscript.

Notes

The authors declare no competing financial interest.

All data generated or analyzed during this study are included in this published article and its supplementary information files.

This article does not contain any studies with animals performed by any of the authors.

We authorize to publish the article without any conflict.

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